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Impact of Therapeutic Treatment with β-Lactam on Transfer of the *bla*$_{\text{CTX-M-9}}$ Resistance Gene from *Salmonella enterica* Serovar Virchow to *Escherichia coli* in Gnotobiotic Rats[▽]

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The conjugative transfer of the plasmid carrying the *bla*$_{\text{CTX-M-9}}$ gene from *Salmonella enterica* serovar Virchow isolated from a chicken farm to a recipient *Escherichia coli* strain was evaluated in vitro and in axenic rats inoculated with both strains, with or without selective pressure due to therapeutic doses of cefixime. The transfer of the *bla*$_{\text{CTX-M-9}}$ gene of *S. enterica* serovar Virchow to *E. coli* was confirmed in vitro, at a low frequency of 5.9×10^{-8} transconjugants/donors. This transfer rate was higher in gnotobiotic rats and reached $\sim 10^{-5}$ transconjugants/donors without selective pressure. This frequency was not affected by the addition of therapeutic doses of cefixime. Thus, estimates of in vitro transfer underestimated potential transfer in the digestive tract, and therapeutic doses of cefixime did not increase the selection for transconjugants.

β-Lactams and extended-spectrum cephalosporins are widely used in human and veterinary medicine to treat severe infections in humans and animals caused by *Enterobacteriaceae* and other gram-negative pathogens (7). However, extended-spectrum β-lactamases (ESBLs) have emerged and become the major mechanism of resistance to β-lactam antibiotics. Until the late 1990s, TEM and SHV enzymes were the predominant ESBLs. However, over the last decade, CTX-M-type enzymes have become the most prevalent extended-spectrum β-lactamases worldwide. Currently, there have been a number of reports documenting an increasing prevalence of enteric pathogens that produce these plasmid-mediated CTX-M enzymes (4, 8, 9, 39).

Although originally confined to hospitals, ESBL-producing strains are now emerging in the community. Several investigations have shown that the rate of fecal carriage of ESBL-positive isolates (especially *Escherichia coli*) in humans is increasing, with CTX-M type enzymes found in most isolates (14, 17, 25, 28, 30, 36). This alarming phenomenon may have serious economic consequences and implications for treatment.

bla$_{\text{CTX-M}}$ genes spread throughout the community, mostly through the transmission of plasmids, and some studies have reported that animals may serve as a possible source for the dissemination of ESBL-encoding genes to humans. Indeed, common conjugative resistance plasmids and resistant clones have been found in animals, food products, and humans, suggesting that the transfer of extended-spectrum cephalosporin resistance between animals and humans is possible (17, 18, 21, 31, 40).

In France, the emergence of the CTX-M-9 enzyme in *Salmonella enterica* serovar Virchow strains recovered from poultry, poultry products, and one human patient was reported between 2002 and 2003 (5, 31, 38, 40, 41). A comparative analysis of the *bla*$_{\text{CTX-M-2}}$ and *bla*$_{\text{CTX-M-9}}$ plasmids from *S. enterica* serovar Virchow isolates from human and poultry sources demonstrated a close relationship between the plasmids (16).

To examine the hypothesis that antimicrobial resistance in humans could partly be attributed to food products, a number of studies have investigated the transfer of antibiotic resistance from bacteria originating in animal hosts or food products. Previously, Lester et al. (24) demonstrated the transfer of the *vanA* resistance gene from *Enterococcus faecium* isolated from animals to *E. faecium* isolated from human volunteers during transient intestinal colonization in humans in the absence of selective pressure. In a gnotobiotic mouse model, Feld et al. demonstrated the evidence of spread of a small plasmid pLFE1 harboring the erythromycin-resistant gene *erm*(B) from *Lactobacillus plantarum* isolated from raw-milk cheese to exogenous *Enterococcus faecalis* inoculated into the intestinal flora of mice (16). The transfer rate was enhanced when erythromycin was coadministered. Improvements in our understanding of the in vivo transmissibility and stability of antimicrobial resistance markers and the mechanisms driving horizontal transmission may help to predict the outcome of different strategies for controlling epidemic plasmids.

The aim of the present study was to determine whether the *bla*$_{\text{CTX-M-9}}$ resistance gene could be transferred from an animal *S. enterica* serovar Virchow strain to a commensal *E. coli* strain that originated from the human intestinal tract while under selective pressure from a therapeutic dose of β-lactam. The transfer was investigated by using in vitro mating and a germfree rat model. We also evaluated the impact of the concentration of cefixime, an expanded-spectrum cephalosporin, on the transfer rate.

MATERIALS AND METHODS

Bacterial strains and culture media. The donor strain used in the transfer experiment was *S. enterica* serovar Virchow 3464b, isolated from a chicken farm (40). This strain is resistant to streptomycin, spectinomycin, tetracycline, trimethoprim, sulfonamides, and nalidixic acid and carries the *bla*$_{\text{CTX-M-9}}$ resis-

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tance gene. The $bla_{CTX\text{-}M\text{-}9}$ gene encodes the CTX-M-9 extended-spectrum β-lactamases and is found on a single plasmid (p3464b) larger than 126 kb (40). The recipient *Escherichia coli* J5 strain is resistant to rifampin (rifampicin; MIC ≤ 250 μg/ml) and susceptible to extended-spectrum cephalosporins, including cefixime. Bacterial strains were cultured in brain heart infusion (BHI) broth or agar (AES, Bruz, France) for 18 h (stationary phase) at 37°C. For the in vitro and in vivo transfer experiment, different selective media were used to select and enumerate the donor, recipient, and transconjugant strains. Donor strains were enumerated on Brilliant Green agar (Difco/BD Biosciences, Le Pont de Claix, France) supplemented with 4 μg of cefixime trihydrate (ytterbium III ionophore I; Sigma-Aldrich, Saint-Quentin Fallavier, France)/ml. Recipient strains were counted on Drigalski lactose agar (Fisher-Bioblock Scientific, Illkirch, France) supplemented with 250 μg/ml of rifampin (Sigma-Aldrich). *E. coli* J5(p3464b) transconjugants were counted on Drigalski lactose agar supplemented with cefixime (4 μg/ml) and rifampin (250 μg/ml).

In vitro mating. Transferability of the $bla_{CTX\text{-}M\text{-}9}$ gene between *S. enterica* serovar Virchow and *E. coli* J5 was studied first in three conjugation experiment procedures: (i) in vitro filter mating, (ii) in vitro mating on agar plate without a filter, and (iii) in vitro mating in liquid media. Procedure 2, showing a higher transfer frequency (data not shown), was used to determine the conjugation rate in the following experiments, as described below. One colony of donor and recipient strains was inoculated separately in BHI broth and grown overnight at 37°C. Recipient and donor cultures were diluted 10-fold with fresh BHI broth. The donor strain was cultured at 37°C until it reached the mid-exponential-growth phase (optical density at 600 nm = 0.7), and 1 ml of the donor and 1 ml of the recipient strain were mixed. An aliquot of the mating mixture (200 μl) was spread on the surface of a BHI agar plate. After 24 h of incubation at 37°C, the cells were suspended in 1 ml of BHI broth. A series of 10-fold dilutions of the mating mixture was prepared, and 200-μl aliquots of each dilution were plated on a selective medium. The colonies on the plates were counted after 24 h of incubation at 37°C. Transfer frequencies were calculated by dividing the number of transconjugants by the number of donor colonies. The geometric mean transfer frequencies were calculated from five independent conjugation experiments to represent the ratio of transconjugants per donor.

In vivo mating. The animal experiments were approved and conducted in accordance with French national legislation. Ten female germfree Oncin France strain A rats (Charles River, Arbresle, France) were transferred after their arrival into a sterile Trexler-type plastic film isolator (Esi Flufrance, Massy, France) and randomly assigned to two groups of five animals. Each group was kept in a separate isolator, and each rat was caged individually. The germfree status of the rats was checked and confirmed immediately after their reception and introduction into the isolator and during the period of acclimatization, by culturing fecal samples to test for the aerobic and anaerobic growth of bacteria and yeast. The animals were provided with ad libitum access to a commercial sterilized diet and given ad libitum autoclaved drinking water. The axenic rats were 5 weeks old at the beginning of the experiment. On day 0, all rats were inoculated intragastrically by gavage with 1 ml of an $\sim 10^9$-CFU/ml suspension of the recipient strain *E. coli* J5. One week later, once the establishment of the recipient strain had been demonstrated by recipient strain enumeration as previously described, rats received 1 ml of an $\sim 10^8$-CFU/ml suspension of the donor strain *S. enterica* serovar Virchow 3464b intragastrically. On day 14 after inoculation of *E. coli* J5, five animals were treated orally with cefixime trihydrate (Oroken, 100 mg; Sanofi-Aventis, Paris, France) (4 mg/kg) in water twice daily for 8 days. The antibiotic was reconstituted according to the manufacturer's instructions by adding sterile water to obtain a concentration of 20 g/liter and stored at 21°C for 1 week. The control group of five animals received drug-free water by gavage. Fresh fecal samples were collected from each rat by provoked defecation on days 0, 1, 7, 8, 14, 15, 16, 17, 18, 19, 20, 21, 22, 27, 29, 31, and 34. The samples were homogenized by whirly mixing in sterile water (10%). Serial 10-fold dilutions were prepared in 0.9% NaCl and spread, as described under the "bacterial strains and culture media" subheading, onto appropriate media within dishes supplemented with antibiotics using the automated spiral plater (Interscience, Paris, France). The detection limit of this procedure was $\sim 10^2$ CFU/g of feces.

Three isolates growing on Drigalski lactose agar containing cefixime and rifampin were arbitrarily isolated from each rat during the experiment at days 14, 27, 31, and 34. Altogether, 60 isolates with resistance phenotype *E. coli* J5(p3464b) transconjugant were confirmed as *E. coli* by PCR amplification of the *iudA* gene, as described by Bej et al. (2, 3). Sample DNA preparations were obtained by adding the InstaGene matrix kit (Bio-Rad, Marnes la Coquette, France) to the bacterial suspension, according to the manufacturer's instructions. Supernatant (5 μl) was used as a template for the PCR.

Plasmid DNA was isolated from 12 transconjugants (representing four rats and 3 days of the study) by using a QIAprep Spin Midiprep Kit (Qiagen, Hilden, Germany). PCR assays, using primers targeting the $bla_{CTX\text{-}M\text{-}9}$ gene, were carried out as described by Weill et al. (40), except the annealing conditions were changed to 30 s at 60°C in our experiment. The donor, recipient, and transconjugants obtained in vitro were included as controls for the PCRs.

Twelve isolates were also tested for antibiotic susceptibility to amoxicillin-clavulanic acid, ampicillin, cefotaxime, cefoxitin, ceftazidime, ceftiofur, cefuroxime, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, trimethoprim, and trimethoprim-sulfamethoxazole by using a commercially prepared, dehydrated panel (Trek Diagnostic Systems, Ltd., East Grinstead, West Sussex, United Kingdom) according to the method and interpretative criteria proposed by CLSI or EUCAST (www.eucast.org) (10, 11). Since most ESBLs have MICs above the established clinical resistance breakpoints, the epidemiological cutoff values (threshold value which distinguishes microorganisms with or without an acquired resistance gene, according to EUCAST) were used to classify the susceptibility of isolates to cefotaxime, ceftazidime, ceftiofur, cefoxitin, and cefuroxime. The MIC of cefixime was determined with an Etest strip (AB Biodisk, Solna, Sweden) on Mueller Hinton agar (AES, Bruz, France). The donor, recipient, and in vitro transconjugants were also included. *E. coli* ATCC 25922 was used as a quality control.

Cefixime concentrations in fecal samples were determined by the liquid chromatography-mass spectrometry (LC-MS) method. The antibiotic levels were determined in fecal samples, twice daily on days 14, 15, 16, 17, 18, 19, 20, 21, 22, 27, 29, 31 and 34. Feces (200 mg) were homogenized in sterile water (10%) and centrifuged (6,000 × *g*, 5 min). Supernatant (1 ml) was taken and centrifuged at 20,000 × *g* for 10 min. The resulting supernatant phase was injected onto the LC-MS system for analysis. The chromatographic separation was achieved on a Zorbax ECLIPSE XDB-C_8 column (150 by 2.1 mm [inner diameter, 5 μm]; Agilent, Wilmington, DE) with a 12.5-by-2.1-mm (inner diameter) Zorbax Eclipse XDB-C8 (5 μm) guard column (Agilent), using a mobile phase of 0.3% acetonitrile-formic acid (19:81 [vol/vol]). The liquid flow rate was set at 0.2 ml/min. The column temperature was maintained at 24°C. The mass spectrometer was operated in positive ionization mode. Quantification was performed with selected reaction monitoring (SRM) of *m/z* 454 to *m/z* 348 for cefixime and cephalexin (internal standard), respectively, with a scan time of 0.3 s per transition showing the $[M+H]^+$ product ion spectra of cefixime and cephalexin. The spray voltage was set at voltage capillary 3 and the cone voltage at 30 kV. Nitrogen was used for "nebulization" at a rate of 500 liters/h. The heated capillary temperature was set to 280°C. The lower limit of quantification was 2 mg/kg. The intra-assay coefficients of variation (repeatability) were 10.69 and 1.36% for quality control samples of 2 and 50 mg/kg, respectively. The interassay coefficients of variation (intermediate precision) were 10.69 and 2.67% for the quality control samples of 2 and 50 mg/kg. The area under the curve (AUC_{14-22}) was calculated by the linear trapezoidal method. The mean concentration of cefixime was estimated during treatment between days 14 and day 22 as AUC_{14-22}/T, with T representing the period of time between days 14 and 22.

The statistical analysis was carried out with SYSTAT version 12.0 for Windows. In the in vivo experiments, the numbers of transconjugants, recipients, and donors, with or without antibiotic treatment, were compared by using a two-way analysis of variance with interaction. The experimental unit used was the rat. Repeat measures ($n = 5$) were taken on the rat for each treatment and time. The factors tested were time, treatment, and the time-treatment interaction. Differences were considered significant at P values of <0.05.

RESULTS

In vitro transfer. The mean frequency in five independent experiments was $(5.9 \pm 5.7) \times 10^{-8}$ (Table 1). All of the transconjugants obtained were resistant to cefixime at 8 μg/ml.

In vivo transfer. Before cefixime administration, *E. coli* J5 was allowed to colonize the digestive tracts of all axenic rats, and the densities of recipient *E. coli* were $\sim 10^9$ CFU per g of feces before donor *S. enterica* serovar Virchow administration (Fig. 1). In all animals, after inoculation with the donor strain, the densities of this bacterium reached $\sim 10^8$ CFU/g of feces, whereas *E. coli* dropped 2 log units from day 0 to day 14. At the same time, *E. coli* J5(p3464b) transconjugants rapidly appeared to reach $\sim 10^3$ CFU/g of feces. For the control treatment, donor, recipient, and transconjugant strains established

TABLE 1. Number of transconjugants per donor in vitro and in gnotobiotic rats treated with or without cefixime

Group[a]	No. of transconjugants ± SD per donor		
	In vitro	In vivo	
		Before treatment	After treatment
Control	$(5.9 \pm 5.7) \times 10^{-8}$	$(5.4 \pm 7.1) \times 10^{-5}$	$(6.3 \pm 1.2) \times 10^{-5}$
Treated		$(1.1 \pm 1.3) \times 10^{-4}$	$(1.2 \pm 5.6) \times 10^{-5}$

[a] Rats treated without cefixime (control) received drug-free water. Treated rats were given 4 mg/kg twice daily for 8 days.

stable population levels throughout the experiment with a mean transconjugant/donor ratio of $\sim 10^{-5}$ (Table 1).

One day after cefixime administration, the number of transconjugants decreased statistically ($P < 0.001$) to undetectable levels. Five days after cefixime treatment (day 27), counts of transconjugants were $\sim 10^3$ CFU/g of feces. By day 35, this population had reached $\sim 10^5$ CFU/g of feces. Conversely, the donor strains shown to be less affected by cefixime treatment than the transconjugant strains, displaying a decrease in frequency by a factor of about 200 over the first few days of treatment. The recipient population dropped drastically by a factor of about 4,000 within 24 h after cefixime administration ($P < 0.001$) and remained at this level throughout the treatment (Fig. 1b). When cefixime was removed, this level was estimated at $\sim 10^4$ CFU/g of feces, with a value of $\sim 10^7$ CFU/g of feces at the end of the experiment. At the end of the experiment, the mean ratio of transconjugant per donor was $(6.3 \pm 1.2) \times 10^{-5}$ and $(1.2 \pm 5.6) \times 10^{-5}$ for the control and treated group, respectively (Table 1).

Verification of transconjugants. PCR analysis indicated the presence of the $bla_{\text{CTX-M-9}}$ gene in all transconjugants.

Table 2 compared the susceptibility of representative transconjugants obtained in vivo before, during, and after cefixime treatment to the susceptibility of donor, recipient, and transconjugants obtained in vitro. Regardless of the sampling treatment period, the transconjugants had acquired resistance to amoxicillin-clavulanic acid, ampicillin, cefixime, cefotaxime, ceftazidime, ceftiofur, and cefuroxime absent from the parental *E. coli* J5 strain. Their susceptibility to cefotaxime, ceftazidime, and ceftiofur had decreased to levels similar to those in *S. enterica* serovar Virchow based on the MIC of these cephalosporin antibiotics and resistance phenotype profiles. Transconjugants were considered to be ESBL-positive strains at the epidemiological EUCAST cutoff value.

Other resistance determinants, including streptomycin, tetracycline, trimethoprim, sulfamethoxazole and trimethoprim-sulfamethoxazole were cotransferred with cephalosporin resistance to in vitro transconjugants but not to transconjugants obtained in vivo. Furthermore, after cefixime treatment, these isolates showed decreased susceptibilities to sulfamethoxazole with MICs of 8 to 32 μg/ml, but not resistance. Nalidixic acid resistance was not transferred to transconjugants obtained in vitro or in vivo.

Quantification of cefixime in rat feces. The cefixime concentrations in the fecal samples from treated rats were estimated by LC-MS, twice daily for 20 days (Fig. 2). Mean cefixime levels were 37.2 mg/kg of feces during cefixime treatment and decreased to reach the limit of quantification of 2 mg/kg in 8 days.

DISCUSSION

This study was prompted by the demonstration that *S. enterica* serovar Virchow isolated from chickens could transfer the $bla_{\text{CTX-M-9}}$ resistance gene to *E. coli* in a conjugation experiment (40). In this prior report by Weill et al., in vitro mating was carried out on liquid media to show the ability of plasmid transfer without indicating the transfer frequency. We have redesigned the experiment in vitro to achieve a transfer rate comparable to that obtained in vivo. Efficient transfer of this gene from the donor to plasmid-free recipients was measured at a frequency of about $(5.9 \pm 5.7) \times 10^{-8}$ transconjugants per donor. This transfer rate is lower than those reported in previously published in vitro mating studies (23, 24). However, it has also been reported that, in vitro, interspecies transfer is less efficient than intraspecies transfer (19, 20, 29).

We investigated whether conjugative transfer of the $bla_{\text{CTX-M-9}}$ gene could also occur in vivo, by carrying out transfer experiments in gnotobiotic rats. This model has already been used to study conjugal transfer, because the use of axenic animals inoculated with only the donor and recipient strains overcomes the problem associated with bacterial competition and promotes intestinal colonization by the strains involved in gene exchange (15). In vivo, *E. coli* transconjugants appeared rapidly in all animals after inoculation with the recipient and donor strains, reaching levels of $\sim 10^5$ CFU/g. These levels remained stable for the 27 days of the experiment in the con-

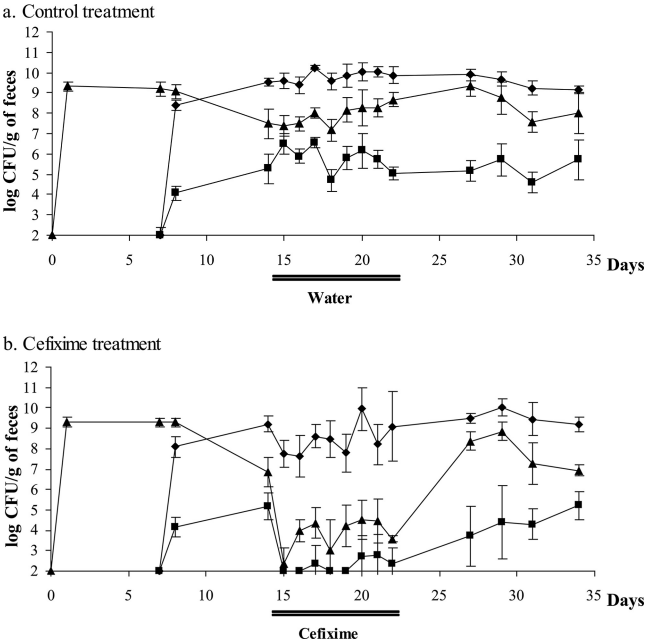

FIG. 1. Bacterial counts in homogenized feces from gnotobiotic rats treated with sterile water ($n = 5$) (control treatment [a]) or with cefixime ($n = 5$) (cefixime treatment [b]). Symbols: ◆, donor *S. enterica* Virchow 3464b; ▲, recipient *E. coli* J5 and *E. coli* J5(p3464b) transconjugants; ■, *E. coli* J5(p3464b) transconjugants. The bar under the *x* axis represents the time of treatment. Values are the means of results, and error bars represent the standard deviation.

TABLE 2. Susceptibility or resistance of the isolated strains in the present study

Antibiotic	Note[a]	Susceptibility (S) or resistance (R) (MIC [μg/ml])						
		E. coli ATCC 25922	Donor *S. enterica* serovar Virchow 3464b	Recipient *E. coli* J5	*E. coli* J5(p3464b) transconjugant in vitro[b]	*E. coli* J5(p3464b) transconjugant[b]		
						Before treatment	7 days posttreatment	12 days posttreatment
Amoxicillin-clavulanic acid	A, D	S (4)	R (8)	S (4)	R (8)	R (32)	R (32)	R (32)
Ampicillin	A, D, E	S (4)	R (128)	S (4)	R (128)	R (64)	R (32)	R (32)
Cefixime	B, D, E	S (0.13)	R (8)	S (0.13)	R (8)	R (8)	R (8)	R (8)
Cefotaxime	A, C, E	S (0.03)	R (>8)	S (0.03)	R (8)	R (0.50)	R (0.50)	R (1)
Cefoxitin	A, C	S (4)	S (16)	S (4)	S (4)	R (32)	S (16)	S (16)
Ceftazidime	A, C, E	S (0.13)	R (8)	S (0.13)	R (2)	R (4)	R (2)	R (2)
Ceftiofur	A, C, E	S (0.25)	R (>8)	S (0.25)	R (8)	R (1)	R (1)	R (1)
Cefuroxime	A, C, E	S (2)	R (>32)	S (2)	R (32)	R (32)	R (32)	R (32)
Nalidixic acid	A, D	S (4)	R (256)	S (4)	S (4)	S (4)	S (8)	S (8)
Streptomycin	A, C	S (2)	R (128)	S (2)	R (16)	S (2)	S (2)	S (2)
Sulfamethoxazole	A, D	S (8)	R (>512)	S (8)	R (>512)	S (8)	S (32)	S (32)
Tetracycline	A, D	S (1)	R (64)	S (1)	R (16)	S (1)	S (2)	S (2)
Trimethoprim	A, D	S (0.50)	R (>64)	S (0.50)	R (16)	S (0.50)	S (1)	S (1)
Trimethoprim-sulfamethoxazole	A, D	S (<1)	R (>16)	S (<1)	R (16)	S (1)	S (1)	S (1)

[a] A, the MICs of antimicrobial agents were determined by the broth microdilution method; B, the MICs of cefixime were determined with Etest strips; C, results were interpretative of susceptible or resistant strains according to the criteria proposed by EUCAST; D, results were interpretative of susceptible or resistant strains according to the criteria proposed by CLSI; E, resistance to this antibiotic was presumably mediated by CTX-M-9 enzyme.

[b] The MICs of one of the four isolated transconjugants are presented in the table (all transconjugants have the same susceptibility values).

trol group. Our results are consistent with other demonstrations of the conjugal transfer of resistance genes in gnotobiotic animals in the absence of selective pressure (12, 18). In vivo, the ratio of transconjugants per donor was 4 log units higher than in mating experiments on an agar plate. Similar findings were recently reported by Feld et al., who obtained a rate of plasmid transfer from *L. plantarum* to *E. faecalis* 3 to 4 log units higher in the gnotobiotic model than in vitro (15). This may be due to the constant mixing of bacteria by the peristaltic movements of the gastrointestinal tract, providing donor bacteria with greater access to recipient bacteria than during filter mating, in which the bacteria move much less (26). Antibiotics have been shown to stimulate the spread of conjugative antibiotic resistance plasmids by increasing transfer rates (35). However, in our study, cefixime treatment at a dose of 4 mg/kg/day twice daily for 8 days was bactericidal rather than exerting selective pressure for both cefixime-susceptible recipients and cefixime-resistant transconjugants and donors. This finding is consistent with the results of a study demonstrating no increase in the rates of transfer of the conjugative plasmid containing *cmy-2* to *S. enterica* Newport and *E. coli* in animals treated with a single dose of ceftiofur (38). Moreover, the apparent antibiotic concentrations measured in feces were 4 to 250 times higher than cefixime MICs for *S. enterica* serovar Virchow (MIC < 8 μg/ml), transconjugant *E. coli* J5 p3464b (MIC < 8 μg/ml), and *E. coli* J5 (MIC < 0.125 μg/ml). Binding to the fecal material has not been determined. However, considering that (i) cefixime is not metabolized by the rat's intrinsic metabolism and (ii) minimal binding in feces is equal to the plasma binding protein, i.e., 63% for an average fecal concentration of 37 μg/g, the actual active concentration of cefixime was 13.7 μg/g, which is much higher than the MIC of susceptible or resistant strains. In cases where binding is 99%, this active concentration will decrease to 0.37 μg/g of feces, which is still twice the MIC of susceptible *E. coli* strains. The decrease in population size observed for all strains was more pronounced for transconjugants (~3.5 log units) than donors (~1.5 log units). After the first administration of cefixime, the levels of donor and recipient strains decreased, whereas transconjugants were more strongly affected by the drug. A possible explanation for this could be that the regrowth of recipient strains after 1 day of cefixime administration was not sufficient to allow plasmid transfer. Donor and recipient strain concentrations must be equivalent or close to provide effective conjugation, and that was not the case in the treated rats (1). Alternatively, donor and transconjugant population levels may affect the MIC of cefixime in the digestive tract. Inoculum size is known to affect the MIC of extended-spectrum cephalosporins, with 40 to 200 times higher MICs reported for the highest levels of inoculum under laboratory conditions (6, 37). This may result in lower rates of multiplication of the transconjugants than of the susceptible parental strain (22). Indeed, the generation time of the *E. coli* recipient strain was 24 min, whereas that of the transconjugants was 38 min (data not shown). These findings are also consistent with the slower increase in the numbers of resistant *E. coli* after the removal of cefixime.

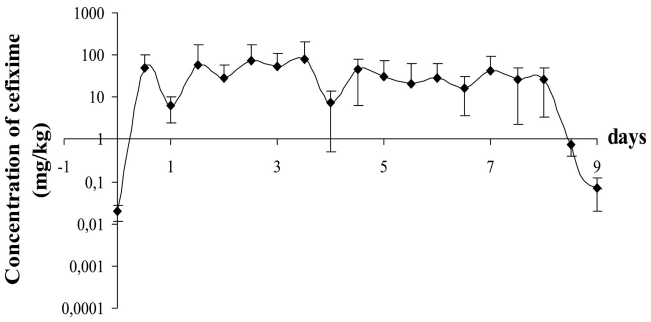

FIG. 2. Mean cefixime concentrations in fecal samples from treated rats ($n = 5$). The errors bars represent the standard deviation.

Nalidixic acid resistance was not transferred in vitro and in

vivo to *E. coli* recipient strains. This result may be attributed to the chromosomic resistance by mutations in the chromosome (40). Resistance to streptomycin, tetracycline, trimethoprim, and trimethoprim-sulfamethoxazole, which were located on single plasmids in the donor strain, were observed in CTX-M-9-producing transconjugants obtained in vitro but not expressed in CTX-M-9-producing transconjugants isolated during the experimental period. Similar results were obtained by Novais et al. (34) for a percentage of transconjugants, even though In*60*-contains the $bla_{\text{CTX-M-9}}$ gene and gene cassettes presumptively responsible for these resistance phenotypes. Hypotheses, such as gene inactivation or silencing, have been put forward, but no conformational experiments have been conducted.

Although antibiotic selective pressure has been shown to increase the proportion of antibiotic resistance genes in vivo, most experiments have been conducted with subinhibitory antibiotic concentrations (13, 27, 32, 33). In our study, we investigated modulation of the transfer during a therapeutic antibiotic treatment. We found that experiments assessing transfer in vitro underestimated potential transfer in the digestive tract. We also showed that transfer of the $bla_{\text{CTX-M-9}}$ resistance gene plasmid occurred in vivo but was not enhanced by cefixime administered at concentrations equivalent to those used in therapeutic treatment. Given the potential risk to humans of the presence of resistant strains in the digestive tract, the antimicrobial treatment effect on resistance gene dissemination should be studied in more detail in a rat model with complex human intestinal flora.

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